Preclinical Evaluation of Nilotinib Efficacy in an Imatinib-Resistant KIT-Driven Tumor Model

Carleen Cullinane1, Anthony Natoli1, Yorton Hui1, Nelly Conus1, Susan Jackson1, Joseph Brüggen3, Paul W. Manley3, and Grant A. McArthur1,2

Abstract

The novel KIT inhibitor nilotinib is currently being evaluated for its clinical utility in the treatment of gastrointestinal stromal tumor. However, the effects of nilotinib in cells expressing commonly occurring KIT mutations remain to be fully defined. The aim of this study was therefore to investigate the efficacy of nilotinib against cells expressing imatinib-sensitive or imatinib-resistant KIT mutations and to evaluate [18F] fluoro-deoxyglucose-positron emission tomography (FDG-PET) imaging as a biomarker of nilotinib response in vivo. Nilotinib inhibited the proliferation of imatinib-responsive V560G-KIT FDC-P1 and imatinib-resistant D816V-KIT FDC-P1 cells with a GI50 of 4.9 and 630 nmol/L, respectively, whereas apoptosis studies revealed that nilotinib and imatinib were equipotent against the V560G cell line. In contrast, although 10 μmol/L nilotinib induced >50% apoptosis in the D816V cells at 16 hours, 10 μmol/L imatinib had no effect on cell survival at 24 hours. Syngeneic DBA2/J mice bearing FDC-P1-KIT tumors were evaluated for response to nilotinib by FDG-PET. V560G-KIT FDC-P1 tumor FDG uptake was significantly reduced compared with baseline levels following 2 days of nilotinib treatment. In contrast, no effect of nilotinib was observed on tumor growth or FDG-PET uptake into D816V tumors despite intratumoral drug levels reaching in excess of 10 μmol/L at 4 hours after dosing. Biomarker analysis revealed the inhibition of KIT phosphorylation in V560G but not D816V tumors. These findings show the in vivo activity of nilotinib in the treatment of tumors bearing V560G-KIT but not D816V-KIT and the utility of FDG-PET imaging to assess tumor response to this agent.

Mol Cancer Ther; 9(5); 1461–8. ©2010 AACR.

Introduction

Activating mutations of the KIT receptor tyrosine kinase occur in >85% of gastrointestinal stromal tumors (GIST), as well as in a range of other human malignancies (1, 2). In GIST, such mutations activate signaling pathways associated with cell proliferation and survival, including the phosphoinositide 3-kinase and mitogen-activated protein kinase pathways (3–5). These tumors are refractory to conventional chemotherapy and radiotherapy and, until recently, the prognosis for GIST patients was very poor, with a median survival of 19 months (6). The small-molecule tyrosine kinase inhibitor imatinib however has revolutionized the treatment of this disease with >85% patients initially responding to treatment (7, 8) and median survival increasing to 57 months (9). GIST response to imatinib is correlated with its KIT genotype. The most common KIT mutations occur in exon 11 (~70% of cases) and are highly responsive to imatinib, whereas exon 9 mutations are less frequent (~15% of cases) and less responsive (10–12). Mutations in exon 13 and 17, although rare in primary tumors, are generally imatinib resistant (11, 13, 14).

Although imatinib therapy has led to significant improvements in survival for GIST patients, many tumors that respond initially will relapse due to acquired drug resistance (15, 16). As the major mechanism of imatinib resistance is the development of secondary KIT mutations, predominantly in the tyrosine kinase domain, the development of novel agents with activity against such mutations is essential to improve patient outcomes.

Nilotinib is a novel phenylaminopyrimidine derivative with potent activity against the BCR-ABL, KIT, DDR, and PDGFR kinases and is currently approved for the treatment of chronic myeloid leukemia (17–19). Nilotinib is well tolerated in patients and has been shown to have activity in imatinib- and sunitinib-resistant GIST (20, 21). However, the characterization of the effects of nilotinib in cells expressing commonly occurring KIT mutations...
is incomplete (22–24) and effects elicited through KIT inhibition in vivo have not been explored.

We have recently developed a murine model of KIT-driven neoplasia suitable for \(^{18}F\) fluorodeoxyglucose-positron emission tomography (FDG-PET) imaging. Using this model, we showed that imatinib causes a rapid reduction in glucose metabolism in FDC-P1 cells expressing V560G-KIT mutation. Furthermore, this change was associated with a reduction in the expression of the glucose transporter GLUT1 and these effects preceded any change in tumor size (14). The aims of the current study were therefore to use the preclinical model to investigate the efficacy of nilotinib in vitro and in vivo against imatinib-sensitive and imatinib-resistant tumors and evaluate FDG-PET imaging as a biomarker of nilotinib response in vivo.

Materials and Methods

Nilotinib and imatinib mesylate were kindly provided by Novartis Pharma. FDG was purchased from Cyclotek. V560G-KIT FDC-P1, D816V-KIT, wild-type (WT)-KIT, and parental FDC P1 cells were kindly provided by Dr. Leonie Ashman (University of Newcastle, Newcastle, Australia). EPOR(R129C) cells (EPOR) were generated by infecting FDC-P1 cells with MSCVcEPOR(R129C) retrovirus as described by Pharr et al. (25).

V560G-KIT+, D816V-KIT+, and cEPOR- FDC-P1 cells were maintained as a suspension culture in DMEM supplemented with 5% fetal bovine serum in a humidified atmosphere of 5% CO\(_2\) in air. Human WT-KIT FDC-P1 cells were maintained in the same medium and supplemented with either IL3 (as described by Jans et al., ref. 26) or 100 ng/mL SCF (Amgen, Inc.). Parental FDC-P1 cells were grown in DMEM supplemented with 10% heat-inactivated fetal bovine serum, IL3, 1 mmol/L sodium pyruvate, 1 mmol/L asparagine, and 0.1 mmol/L β-mercaptoethanol. All cell lines were routinely tested and confirmed to be Mycoplasma free.

**Cell proliferation studies.** Cells were incubated for 24 hours in complete medium containing up to 10 μmol/L of drug. Viable cells, based on cell size, were then counted using a CDA-500 cell counter (Sysmex Corp.). Duplicate samples at each drug concentration were averaged and cell proliferation then expressed as a percentage of cells in the vehicle control samples.

**Cell viability studies.** Cells were seeded into plates for 24 hours before medium-containing drug was added and the cells were incubated for a further 24 hours. Cells were then harvested, washed twice in PBS containing 2% fetal bovine serum, and then pelleted. The cell pellets were resuspended in 10 μg/mL propidium iodide and 0.25 mg/mL RNase A in PBS, incubated for 30 minutes at 37°C before the cells were analyzed on a fluorescence-activated cell sorter Canto II flow cytometer (Becton Dickinson) for sub-G\(_1\) DNA content. Analysis was done using the FCS Express software.

**Glucose uptake studies.** Drug dilutions were added to plates containing cells incubated overnight and the cells were then allowed to incubate for a further 24 hours. The cells were then washed in glucose-free DMEM (Life Technologies) and pelleted. The pellet was resuspended in a glucose-free medium containing 100 μmol/L 2-deoxy-D-glucose and 0.5 μCi 2-deoxy-D-\(^{1-3}H\)glucose (1 μCi/μL, 6 Ci/mmol, Amersham) and incubated at 37°C for 10 minutes before phloretin (0.6 mmol/L) was added. The samples were washed and the cells lysed in 0.1 mol/L NaOH and 2% Triton X-100. Radioactivity was determined by scintillation counting in Count Safe (Becton Dickinson) and results were standardized for protein content in each sample.

**Western blots.** Cell lysates were prepared in SDS lysis buffer [100 mmol/L Tris-HCl (pH 6.8), 2% SDS, 20% glycerol and 1 mmol/L DTT] and the protein concentration of the resulting supernatants was determined using a Lowry protein assay kit (Bio-Rad). Fifty micrograms of whole protein were loaded onto a sodium dodecylsulfate-polyacrylamide gel and electrophoresed. Western blotting was then done using standard techniques. Antigens were detected with fluorescently labeled secondary antibodies, goat anti-rabbit Alexa Fluor 680 IgG (Molecular Probes), and anti-mouse IRDye 800 IgG (Rockland Immunocamicals) using the Odyssey Infrared Detection System (LI-COR). Antibodies were used at 1:1,000 unless otherwise indicated: phospho-KIT (Y823; Novus NSB 498), c-KIT (DAKO; accession no. A4502), phospho-Akt (Ser473; Cell Signaling Technology; accession no. 9271), phospho-p44/42 MAP kinase (Thr202/Tyr204; Cell Signaling Technology; accession no. 9101), phospho-4E-BP1 (Thr37/46; Cell Signaling Technology; accession no. 2855), phospho-Stat3 (Ser727; Cell Signaling Technology; accession no. 9134) and actin (MP Biomedicals; accession no. 69100; 1:20,000).

**In vivo experiments.** Mice were purchased from the Animal Resources Centre, Western Australia. All in vivo experiments were done according to the National Health and Medical Research Council (NHMRC) Australian Code of Practice or the Care and Use of Animals for Scientific Purposes (7th edition) and with approval from the institutional animal experimentation ethics committee.

FDC-D816V-KIT cells (2 × 10\(^3\)) and FDC-V560G-KIT cells (1 × 10\(^3\)) in 50% Matrigel (Becton Dickinson) were implanted s.c. onto opposing flanks of syngeneic DBA/2J mice. Approximately 2 weeks later when the tumors were ∼200 mm\(^3\) as determined by calliper measurements, mice were randomized into two groups and treated with vehicle or drug as appropriate. Imatinib mesylate was given orally in sterile Milli H\(_2\)O, twice daily at 100 mg/kg, and nilotinib (as hydrochloride salt) was given orally at 100 mg/kg once daily in 10% N-methylpyrrolidinone, 90% polyethylene glycol 300.

For FDG-PET imaging experiments, a baseline PET scan was done the day before drug treatment (day 1). Briefly, animals fasted for 3 hours and then were anesthetized with isoflurane and injected i.v. with 15 MBq FDG.
Anesthesia was maintained for a further 15 minutes before the animals were allowed to recover. Ninety minutes after the tracer injection, the animals were anaesthetized again, imaged, then analyzed on a Philips Mosaic small animal PET scanner as previously described (14). Drug dosing commenced on day 2 and mice were scanned either 4 hours following a single drug dose on day 2 or 4 hours following dosing on day 3. PET scanning was done as previously described (14, 27). After day 2 or 3 imaging, the animals were sacrificed and tumors were removed and bisected. One half was fixed in formalin and the other half was snap frozen in liquid nitrogen. The frozen tumors were crushed in cryogenically cooled stainless steel mortar and pestle, and the cells were lysed and protein extracted as described above.

**Intratumoral drug levels.** Peripheral blood was taken from anaesthetized animals into heparinized tubes and plasma was then prepared and stored at −80°C until analysis. Following sacrifice, the tumors were excised, weighed, snap frozen in liquid nitrogen, and stored at −80°C until analyzed. Concentrations of nilotinib in plasma and tissue were determined by high-pressure liquid chromatography/tandem mass spectrometry, following reported procedures (17), and modified accordingly. Briefly, following chromatographic separation, the column eluent was directly introduced into the ion source of the triple quadrupole mass spectrometer Quattro Ultima (Micromass) controlled by the Masslynx 4.0 software. Electrospray-positive ionization multiple reaction monitoring was used for the tandem mass spectrometry detection of the analyte nilotinib. Precursor to product ion transitions of m/z 530.25 → m/z 289.10 for nilotinib and m/z 516.20 → m/z 289.05 for the structurally related internal standard were used. The limit of quantification was set to 4 ng/mL and 4 ng/g for plasma and tissues, respectively (coefficient of variation and overall bias, <30%). Regression analysis and further calculations were done using QuanLynx 4.0 (Micromass) and Excel 2002 (Microsoft). Concentrations of unknown samples were back calculated based on the peak area ratios of analyte/internal standard from a calibration curve constructed using calibration samples spiked in blank plasma or tumor tissue obtained from nontreated (not dosed) animals. Assay linearity was indicated by an overall regression coefficient of 0.9969.

**Results**

Initial studies were done to evaluate the in vitro activity of nilotinib against the murine leukemia cell line expressing the imatinib-sensitive V560G-KIT and imatinib-resistant D816V-KIT mutations. A panel of control cell lines was included to confirm the specificity of any effects observed. Control cell lines used included parental FDC-P1s supplemented with IL3, parental cells expressing human WT-KIT and supplemented with stem cell factor or IL3, respectively, and FDC-P1-cEPO-R cells that express a constitutively active erythropoietin receptor and are growth factor independent.

*Nilotinib inhibits V560G-KIT and D816-KIT cell proliferation, viability, and uptake of glucose in vitro.* Cell proliferation assays revealed that the FDC-V560G-KIT cells are highly sensitive to both nilotinib and imatinib with GI50 values of 5 and 6 nmol/L, respectively (Table 1). In contrast, FDC-P1 cells expressing the imatinib-resistant mutation D816V-KIT were 8-fold more sensitive to nilotinib than imatinib. Despite the enhanced antiproliferative activity of nilotinib against the imatinib-resistant cells, these cells remained ~130-fold more resistant to nilotinib than the V560G-KIT-expressing cells. As expected, the control cell lines FDC-cEPO-R, FDC-parental, and FDC-WT-KIT + IL3 were highly resistant to both compounds.

To investigate the effects of nilotinib on cell survival, flow cytometry analysis of DNA content was done over a drug exposure period of 24 hours and the results are shown in Fig. 1. The V560G-KIT cells showed a similar high sensitivity to both nilotinib (Fig. 1A) and imatinib (Fig. 1B) with >50% cell death observed at 16 hours. Treatment of D816V-KIT cells at 10 μmol/L imatinib had no effect on cell survival, whereas at the same dose of nilotinib, a 50% reduction in cell viability was observed at 16 hours. As observed previously (17, 28, 29), nilotinib also exerted antiproliferative effects against WT-KIT–expressing cells treated with stem cell factor (Table 1; Fig. 1A and B).

We have previously shown that response to imatinib in this tumor model is associated with a rapid reduction in cellular glucose uptake (14). We therefore investigated the effects of nilotinib on in vitro glucose uptake (14). The results are shown in Fig. 1 and support the findings obtained in the cell proliferation assays. As expected, nilotinib inhibited cellular glucose uptake in the cell line panel. As expected, both nilotinib and imatinib

<table>
<thead>
<tr>
<th>Drug</th>
<th>V560G</th>
<th>D816V</th>
<th>WT-KIT + SCF</th>
<th>WT-KIT + IL3</th>
<th>Parental</th>
<th>cEPO-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI50 (μmol/L)*</td>
<td>0.0049 ± 0.0005†</td>
<td>0.63 ± 0.06</td>
<td>0.19 ± 0.05</td>
<td>2.8 ± 0.4</td>
<td>2.5 ± 0.6</td>
<td>4.9 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.0057 ± 0.0012</td>
<td>5.2 ± 0.6</td>
<td>0.15 ± 0.02</td>
<td>4.2 ± 0.6</td>
<td>6.4 ± 0.7</td>
<td>4.9 ± 0.6</td>
</tr>
</tbody>
</table>

*Drug concentration required to inhibit cell proliferation by 50%.
†Mean ± SEM of at least three independent experiments.
potently inhibited glucose uptake into V560G-KIT cells (Fig. 1C and D). Although nilotinib exhibited nonspecific effects on glucose uptake, as seen by the dose-dependent reduction in glucose uptake in the control lines (Fig. 1C), uptake into D816V-KIT cells was inhibited by nilotinib to a similar extent as the WT-KIT+SCF cells. In contrast, although imatinib reduced glucose uptake in WT-KIT cells in a dose-dependent manner, no effect was observed in D816V cells up to 2 μmol/L (Fig. 1D), suggesting that nilotinib perturbs glucose uptake in the D816V-KIT cells to a greater extent than imatinib.

The effect of nilotinib on the activation of KIT and downstream signaling pathways was then investigated (Fig. 2). In the V560G-KIT cells, both nilotinib and imatinib treatment abolished the phosphorylation of KIT, extracellular signal-regulated kinase, and the S6 kinase target rpS6, showing substantial shutdown of the KIT, mitogen-activated protein kinase, and mammalian target of rapamycin signaling pathways. In D816V-KIT cells, KIT phosphorylation was strongly inhibited by nilotinib, but only modestly by imatinib. Neither compound however reduced phosphorylation of extracellular signal-regulated kinase and nilotinib, but not imatinib, inhibited phosphorylation of rpS6 to a modest extent. These findings show that nilotinib partly inhibits KIT signaling in the D816V-KIT cells.

Nilotinib induces regression of V560G-KIT tumors but has no effect on D816V-KIT tumor growth. Taken together,
the *in vitro* data suggest that nilotinib has more potent cellular effects than imatinib against the D816V-KIT cells. To investigate whether these findings translate to the *in vivo* setting, we evaluated the effects of the compounds on *in vivo* tumor growth and metabolism.

Syngeneic DBA2/J mice bearing FDC-V560G-KIT or D816V-KIT tumors were treated with 100 mg/kg once or twice daily with nilotinib or imatinib mesylate, respectively. As seen in Fig. 3, the V560G tumors regressed rapidly upon treatment with both nilotinib (Fig. 3A) and imatinib (Fig. 3B), whereas neither compound inhibited D816V-KIT tumor growth. To investigate nilotinib exposure, plasma and intratumoral nilotinib levels were determined 4 hours following a single drug treatment. Consistent with findings in other mouse pharmacokinetic studies with nilotinib (data not shown), plasma levels of >30 μmol/L were achieved (Table 2). The intratumoral nilotinib concentration was in excess of 10 μmol/L, a dose shown by the *in vitro* studies to affect D816V-KIT cell proliferation, survival, and glucose uptake.

**Tumor response to nilotinib is associated with a reduction in fluorodeoxyglucose uptake.** *FDG-PET* imaging studies were done to investigate the effects of nilotinib on FDG uptake into tumors expressing the imatinib-sensitive and imatinib-resistant KIT mutations. Mice were imaged at baseline and either 4 hours after a single drug dose or 4 hours after dosing on the second treatment day. Figure 4A shows representative images of mice from the nilotinib group at these time points. As observed previously (14), both KIT-expressing tumors show avid FDG uptake. Whereas no reduction in FDG uptake into D816V-KIT tumors was observed following nilotinib treatment, a clear reduction in FDG signal was observed in the V560G-KIT tumors at 28 hours. Quantification of tumor-specific uptake revealed that imatinib and nilotinib reduced FDG uptake into V560G-KIT tumors at 4 hours (imatinib, *P* < 0.05) and at 28 hours (both drugs, *P* < 0.05; Fig. 4A and B). No effect of either compound on FDG uptake was observed in the D816V-KIT model (Fig. 4C). Tumors from mice imaged at 4 hours (data not shown) and 28 hours (Fig. 4D) after dosing were evaluated for the effect of each compound on activity of KIT and its downstream signaling pathways. The V560G-KIT tumors showed a reduction in phosphorylated and total KIT at both time points following treatment with either imatinib or nilotinib. In contrast, no effect of either compound was observed in KIT in the D816V tumors, consistent with the absence of *in vivo* activity of the compounds against this KIT mutation.

**Discussion**

Although imatinib has revolutionized the treatment of GIST, acquired drug resistance and, to a lesser extent, imatinib intolerability are ongoing challenges to improving outcomes for patients with this disease. Nilotinib is a second generation inhibitor of the BCR-ABL tyrosine kinase that, like imatinib, also inhibits the kinase activity of KIT and PDGFR A and B. Although nilotinib is well tolerated and has activity in imatinib- and sunitinib-resistant GIST in the clinic (20), the effect of nilotinib on KIT mutations found in GIST remains poorly defined. We therefore used

![Figure 3. Nilotinib inhibits V560G-KIT but not D816V-KIT tumor growth *in vivo*. DBA2/J mice bearing a V560G-KIT and D816V-KIT tumor on opposing flanks were treated orally, once daily nilotinib hydrochloride at 100 mg/kg or drug vehicle (A) or orally twice daily with imatinib mesylate at 100 mg/kg or vehicle (B). Tumor volumes were measured using electronic callipers and tumor volumes are expressed as a ratio of tumor size on day 1. Points, mean of five mice per group; bars, SEM.](https://mct.aacrjournals.org/figs/10.1158/1535-7163.MCT-09-1181/Fig3.jpg)

![Table 2. Plasma and intratumoral concentrations of nilotinib](https://mct.aacrjournals.org/figs/10.1158/1535-7163.MCT-09-1181/Table2.jpg)

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Concentration</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plasma (μmol/L)</td>
<td>Tumor V560G (nmol/g)</td>
<td>Tumor D816V (nmol/g)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>2.91 ± 0.89*</td>
<td>2.51 ± 0.49</td>
<td>3.31 ± 0.90</td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>33.12 ± 2.53</td>
<td>12.79 ± 1.42</td>
<td>16.71 ± 1.61</td>
<td></td>
</tr>
</tbody>
</table>

*Mean ± SEM; *n* = 4 or 9 mice per group for the 10 or 100 mg/kg groups, respectively.*
our well-defined preclinical model of KIT-driven neoplasia to evaluate the effects of nilotinib on defined KIT genotypes both in vitro and in vivo.

In vitro studies were done to characterize the cellular effect of nilotinib on cells expressing the imatinib-sensitive V560G-KIT mutation and the imatinib-resistant D816V-KIT mutation. Cell proliferation, viability, and glucose uptake studies revealed that nilotinib induces very similar effects to imatinib against cells expressing the exon 11 mutation V560G-KIT. These findings were confirmed in the in vivo efficacy studies in which both compounds caused rapid regression of V560G-KIT tumors. Based on these findings, it is likely that nilotinib would be an effective alternative agent to imatinib for treatment of GIST tumors expressing this mutation.

Mutations in the kinase domain of KIT frequently render KIT-dependent cells refractory to targeted kinase inhibitors and this resistance can sometimes be explained on a molecular basis. Crystallographic studies have shown that imatinib binds WT-KIT with the activation loop in an inactive conformation, with the Asp816 carboxylate side chain forming a hydrogen-bond with the amide side chain of Gln819, thereby stabilizing the protein conformation (30, 31). Although no crystal structures have been solved for inhibitors in complex with the exon 11, V560G-KIT mutation, it can be speculated that this mutation would not greatly affect the binding mode of imatinib because it is located in the juxtamembrane domain of the protein and is remote from the catalytic domain where the drug binds. However, in the case of the exon 17, D816V-KIT mutation, the replacement Val residue is unable to make the interaction with Gln819, which results in the inactive conformation being destabilized in favor of the active KIT conformation that does not bind either imatinib or sunitinib, rendering the mutant enzyme less sensitive to these inhibitors. Based on the binding of nilotinib to ABL (17), it is expected that nilotinib also binds to KIT in a similar mode to that of imatinib, although the degree of sensitivity of cells expressing the D816V-KIT mutation toward nilotinib remains to be fully characterized.
Intriguingly, von Bubnoff et al. (32) showed that nilotinib inhibited proliferation and induced apoptosis in Ba/F3 cells expressing the murine mutation equivalent to the human D816V-KIT mutation. These findings were supported by several other in vitro studies that showed antiproliferative effects of nilotinib in other models expressing this mutation (28, 30). The in vitro results obtained using the FDC-P1 model were also in agreement with these previous findings. Nilotinib inhibited cell proliferation at submicromolar doses and affected cell viability and KIT signaling pathways, albeit incompletely at 7 to 10 μmol/L.

The in vitro effects of nilotinib against the D816V-KIT mutation however did not translate into in vivo activity, despite plasma and intratumoral drug levels exceeding 30 and 10 μmol/L, respectively, at 4 hours postdosing. Although crystallographic studies would be required to establish the nature of any complex formed between nilotinib and the D816V-KIT mutant protein, the apparent discrepancy between the in vitro and in vivo findings in the D816V-KIT cells may reflect incomplete KIT pathway inactivation in vivo. Indeed, the plasma concentration of nilotinib was only 3-fold higher than that shown in vitro to partially modulate D816V-KIT cellular effects. As the pharmacodynamic effects of nilotinib in vivo are likely to depend on both the extent and duration of KIT kinase inhibition, incomplete kinase inhibition at doses achievable in vivo, coupled with the short half-life of the compound in mice (4 h in OCI mice), suggests nilotinib levels in vivo are insufficient to modulate KIT signaling sufficiently in the D816V-KIT cells for a biological effect. In contrast, although 20 nmol/L nilotinib has profound cellular effects in vitro against V560G-KIT cells, 1,500-fold higher concentrations were achieved in tumors at 4 hours after in vitro dosing and potent tumor regression was observed.

Previous studies have shown that nilotinib reaches intracellular concentrations up to 7- to 10-fold higher than imatinib in GIST cell lines, presumably due to the reduced affinity of nilotinib for cellular drug efflux pumps (33). Furthermore, the observed synergy between imatinib and nilotinib in BCR-ABL+ leukemia lines (34) is suggested to reflect the enhanced intracellular concentration of nilotinib (35). Further studies may therefore be warranted to evaluate the effectiveness of this drug combination against cells expressing D816V-KIT and determine whether the increased intracellular nilotinib concentrations achieved are sufficient to exert a therapeutic effect against cells expressing this mutation.

Inhibition of oncogenic KIT signaling by imatinib leads to a rapid reduction in membrane localization of the glucose transporter GLUT-1 and a corresponding reduction in FDG uptake (14). FDG-PET imaging is a standard, early predictor of GIST response to imatinib in the clinic. Furthermore, the utility of this modality for the early assessment of response to sunitinib in the imatinib-resistant setting has recently been shown (36). Our results using the preclinical KIT-driven tumor model show that FDG-PET is also a very early and robust marker of tumor response to nilotinib with detection of changes in FDG uptake as early as 4 hours. Thus, early monitoring of nilotinib-treated patients for a PET metabolic response would be expected to identify tumors responding to treatment and facilitate the timely switch of therapy in patients whose tumors do not respond, thereby optimizing patient treatment.

Disclosure of Potential Conflicts of Interest

J. Brüggen and P.A. Manley: employees, Novartis Pharma. No other potential conflicts of interest were disclosed.

Acknowledgments

We thank Alison Slater, Rachael Walker, and Ekaterina Bogatyreva for their technical assistance in performing the cell culture, animal experiments, and PET imaging, respectively.

Grant Support

This work was supported by a grant to G.A. McArthur from Novartis Pharma.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 12/17/2009; revised 03/24/2010; accepted 03/24/2010; published OnlineFirst 05/04/2010.

References


Molecular Cancer Therapeutics

Preclinical Evaluation of Nilotinib Efficacy in an Imatinib-Resistant KIT-Driven Tumor Model

Carleen Cullinane, Anthony Natoli, Yorlon Hui, et al.


**Updated version**  Access the most recent version of this article at:
doi:10.1158/1535-7163.MCT-09-1181

**Supplementary Material**  Access the most recent supplemental material at:
http://mct.aacrjournals.org/content/suppl/2010/05/11/1535-7163.MCT-09-1181.DC1

**Cited articles**  This article cites 36 articles, 22 of which you can access for free at:
http://mct.aacrjournals.org/content/9/5/1461.full#ref-list-1

**Citing articles**  This article has been cited by 1 HighWire-hosted articles. Access the articles at:
http://mct.aacrjournals.org/content/9/5/1461.full#related-urls

**E-mail alerts**  Sign up to receive free email-alerts related to this article or journal.

**Reprints and Subscriptions**  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

**Permissions**  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.